

Applicant : Ulrich Laemmli
Serial No. : 09/892,085
Filed : June 26, 2001
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In the Specification

Please amend the specification under 37 C.F.R. §1.121 as follows:

Please replace the paragraph on page 27, lines 19-25 with the following paragraph:

--Panel B: Proposed 1:1 binding model for the complex of P31 (Im-b-Im-Py-b-Im-b-Im-b-Dp where Im = N-methylimidazole) with AAGAGAAGAG (SEQ ID NO:1). Black balls represent imidazole. Circles containing an H represent the N2 hydrogen of guanine. Dashed lines illustrate putative hydrogen bonds. Consensus binding sequences are indicated.--

Please replace the paragraph on page 36, lines 1-19 with the following paragraph:

--A drawback of this binding model, as opposed to conventional 2:1 drug to DNA complexes, is that P31 is expected to bind degenerate GC and CG base pairs, albeit with different affinity. The consensus sequence can thus be defined as SWSWSWSWW (SEQ ID NO:2), where S stands for a G or C and W for A or T. To evaluate binding of P31 to CACAA repeats, we used a second probe that contains two of these repeats as well as five tandem GAGAA repeats. Figure (3A) shows that P31 protects CACAA repeats with approximately five fold lower affinity than GAGAA repeats (lanes 11-15). Furthermore, affinity cleavage reactions using P31E revealed two major cleavage sites in the GAGAA region (lane 16), showing that in this case, two P31 molecules are bound in tandem to the pentameric GAGAA repeat. Again, it is observed that this molecule binds as a 1:1 drug to DNA complex in an orientation as indicated by arrowheads (Figure 3A). We propose that special

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structural features of AT-tracts and GAGAA repeats might favor 1:1 DNA to drug complexes.--

Please replace the paragraph on page 45, lines 22 to page 46, line 1 with the following paragraph:

--Preparation of probes for DNase I footprinting.

S y n t h e t i c o l i g o n u c l e o t i d e s GATCTAGACGCATATTAATTGCGCTGTCGACGCATTAGTG (SEQ ID NO:3) and GATCCACTAATGCGTCGACAGCGCAATTAATATGCGTCTA (SEQ ID NO:4) were hybridized to obtain the W9 probe, oligomerized by ligation and digested with BamHI and BglII to obtain different tandem repeats. The following oligonucleotides were prepared identically: GAF31 is composed of the oligonucleotides GATCCTCAGAGAGAGCGCAAGAGCGTCCCGGGAGAAGAGAAGAGAGTA (SEQ ID NO:5) and GATCTACTCTTCTTCTTCTCCCGGGACGCTCTTGCGCTCTCTCTGAG (SEQ ID NO:6) and Brown I of oligonucleotides GATCCAAGAGAAGAGAAGAGAAGAGAAGAGTACTTATTAACACAACACA (SEQ ID NO:7) and GATCTTGTGTTGTGTTAATAAGTACTCTTCTTCTTCTTCTTCTTCTTG (SEQ ID NO:8).--

Please replace the paragraph on page 61, lines 1-14, with the following paragraph:

--To test whether P31 binds the GAF consensus sequence (GAGAG), we performed footprinting analysis using a DNA probe that includes, besides 2 consecutive *bw^D* repeats (GAGAAGAGAA) (SEQ ID NO:9), a high affinity binding site for GAF corresponding to the *Ubx* promoter sequence ENRfu (Biggin and Tjian, 1988). Inspection of the footprint data showed that, although P31 protects the *bw^D* repeats at a 0.1 nM concentration, no binding is noted at the *Ubx* GAF site with a ligand concentration up to 25 nM (see Figure 7A, NENRfu (Janssen et al., 2000)). We also studied the interaction of

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GAF with this same DNA probe and observed that this protein, in contrast to P31, protects both the *bw^p* repeats and the *Ubx* site (Figure 2B, ENRfu(Janssen et al., 2000))--